CLAIMS

WE CLAIM:

1. A method for producing a population highly enriched for human central nervous system stem cells (CNS-SC) which can initiate neurospheres (NS-IC), comprising:

   (a) contacting a population containing neural or neural-derived cells with a reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12; and

   (b) selecting for cells in which there is contact between the reagent and the determinant on the surface of the cells of step (a), to produce a population highly enriched for CNS-SC.

2. The method of claim 1, wherein the reagent is an antibody that recognizes a determinant on the cell surface marker recognized by monoclonal antibody AC133.

3. The method of claim 2, wherein the reagent is an AC133 monoclonal or polyclonal antibody.

4. The method of claim 2, wherein the reagent is a ligand or small molecule that binds to the surface marker recognized by an AC133 antibody or a 5E12 antibody.

5. The method of claim 1, wherein the reagent is an antibody that recognizes a determinant on the cell surface marker recognized by a 5E12 monoclonal antibody.

6. The method of claim 5, wherein the reagent is a 5E12 monoclonal or polyclonal antibody.

7. The method of claim 1, wherein the reagent is fluorochrome conjugated.

8. The method of claim 1, wherein the reagent is conjugated to magnetic particles.

9. The method of claim 1, wherein the selecting is by flow cytometry.

10. The method of claim 1, wherein the selecting is by fluorescence activated cell sorting or high gradient magnetic selection.

11. The method of claim 1, wherein the selecting is by a physical positive selection device.

12. The method of claim 1, wherein the population containing neural or neural-derived cells is obtained from any tissue which gives rise to neural tissue.

13. The method of claim 1, wherein the population containing neural or neural-derived cells is dissociated from neural tissue.

14. The method of claim 1, wherein the population containing neural or neural-derived cells is derived from a fetal brain, adult brain, fetal spinal cord or adult spinal cord.

15. The method of claim 1, wherein the population containing neural or neural-derived cells is obtained from a neural cell culture.

16. The method of claim 1, wherein the population containing neural or neural-derived cells is obtained from a neurosphere culture or an adherent monolayer.

17. The method of claim 1, further comprising:

(c) contacting the population containing neural or neural-derived cells with a reagent that binds to CD45 antigens; and

(d) selecting for cells in which there is contact between the cells and the reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12 and selecting for reduced contact between the cells and the reagent that binds to CD45 antigens, such that those cells that are $AC133^+$ $CD45^-$ or $5E12^+$ $CD45^-$ or $AC133^+$ $5E12^+$ $CD45^-$ are selected.

18. The method of claim 1, further comprising:

(c) contacting the population containing neural or neural-derived cells with a reagent that binds to CD45 antigen;

(c) contacting the population containing neural or neural-derived cells with a reagent that binds to CD34 antigen; and

(b) selecting for cells in which there is contact between the cells and the reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12 and a reduced contact between the cells and the reagent that binds to CD45 antigen, and between the cells and the reagent that binds to CD34 antigen, such that those cells that are $AC133^+$ $CD45^-$ $CD34^-$ or $5E12^+$ $CD45^-$ $CD34^-$ or $AC133^+$ $5E12^+$ $CD45^-$ $CD34^-$ are selected.

19. The method of claim 1, further comprising:

(c) contacting the population containing neural or neural-derived cells with a reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody 8G1; and

(b) selecting for cells in which there is contact between the cells and the reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12 and a reduced contact between the cells and the reagent that recognizes a

determinant on a cell surface marker recognized by monoclonal antibody 8G1, such that those cells that are $AC133^+$ $8G1^{-/lo}$ or $5E12^+$ $8G1^{-/lo}$ or $AC133^+$ $5E12^+$ $8G1^{-/lo}$ are selected.

20. A method for enriching a population of neural cells for the population's neurosphere initiating stem cell (NS-IC) fraction, comprising:

(a) combining a population comprising neural cells or neural-derived cells containing a fraction of NS-ICs with a reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12; and

(b) selecting for $AC133^+$ and/or $5E12^+$ cells, wherein the selected $AC133^+$ or $5E12^+$ cells are enriched in the fraction of NS-IC as compared with the population of neural cells.

21. A method for identifying a neurosphere initiating stem cell (NS-IC), comprising:

(a) contacting a population containing neural or neural-derived cells with a reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12; and

(b) detecting the contact between a cell of the population containing neural or neural-derived cells and the reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12, wherein an identification of a cell as being $AC133^+$ or $5E12^+$ identifies the cell as an NS-IC.

22. A method for isolating a neurosphere initiating stem cell (NS-IC), comprising:

(a) combining a population comprising neural cells or neural-derived cells containing a fraction of NS-ICs with a reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12;

(b) selecting $AC133^+$ or $5E12^+$ cells, wherein the selected $AC133^+$ or $5E12^+$ cells are enriched in the fraction of NS-ICs as compared with the population of neural cells;

(c) introducing at least one $AC133^+$ or $5E12^+$ cell to a culture medium capable of supporting the growth of NS-IC; and

(d) proliferating the $AC133^+$ or $5E12^+$ cell in the culture medium.

23. A population of cells produced by the method of claim 22.

24. The method of claim 22, wherein the culture medium capable of supporting the growth of NS-IC comprises a serum-free medium containing one or more predetermined growth factors effective for inducing and/or permitting multipotent neural stem cell proliferation.

25. The method of claim 24, wherein the culture medium capable of supporting the growth of NS-IC further comprises a growth factor selected from the group consisting of leukocyte inhibitory factor (LIF), epidermal growth factor (EGF), basic fibroblast growth factor (FGF-2) and combinations thereof.

26. The method of claim 25, wherein the culture medium capable of supporting the growth of NS-IC further comprises a neural survival factor (NSF).

27. An *in vitro* cell culture composition comprising:

(a) a population enriched in $AC133^+$ $CD45^-$ cells or $5E12^+$ $CD45^-$ cells; and

(b) a medium capable of supporting the growth the cells.

28. An *in vitro* cell culture composition comprising:

(a) a population enriched in $AC133^+$ $CD45^-$ $CD34^-$ cells or $5E12^+$ $CD45^-$ $CD34^-$ cells; and

(b) a medium capable of supporting the growth the cells.

29. An *in vitro* cell culture composition comprising:

(a) a population enriched in $AC133^+$ $8G1^{-/lo}$ cells or $5E12^+$ $8G1^{-/lo}$ cells; and

(b) a medium capable of supporting the growth the cells.

30. An *in vitro* cell culture composition comprising:

(a) a population enriched in $AC133^+$ $8G1^{hi}$ cells or $5E12^+$ $8G1^{hi}$ cells; and

(b) a medium capable of supporting the growth the cells.

31. An *in vitro* cell culture composition comprising:

(a) a population comprising at least 50% $AC133^+$ or $5E12^+$ neurosphere initiating cells (NS-IC) which stain positive for nestin and, in the presence of differentiation-inducing conditions, produce progeny cells that differentiate into neurons, astrocytes, and oligodendrocytes; and

(b) a medium capable of supporting the growth of NS-IC.

32. The composition of claim 31, further comprising a solid support to which the NS-IC are attached.

33. The composition of claim 31, wherein the population of cells has at least 70% $AC133^+$ or $5E12^+$ cells.

34. The composition of claim 31, wherein the population of cells has at least 90% $AC133^+$ or $5E12^+$ cells.

35. The composition of claim 31, wherein the population of $AC133^+$ or $5E12^+$ cells is a substantially pure population.

36. The composition of claim 31, wherein the medium comprises a serum-free medium containing one or more predetermined growth factors effective for inducing multipotent neural stem cell proliferation.

37. The composition of claim 31, wherein the medium further contains a growth factor selected from the group consisting of leukocyte inhibitory factor (LIF), epidermal growth factor (EGF), basic fibroblast growth factor (FGF-2), and combinations thereof.

38. The composition of claim 31, wherein the medium further comprises a neural survival factor.

39. The composition of claim 31, wherein the NS-IC are human.

40. A method for characterizing a neurosphere initiating stem cell (NS-IC), comprising:

(a) introducing an isolated $AC133^+$ or $5E12^+$ cell to a culture medium capable of supporting the growth of NS-IC;

(b) proliferating the $AC133^+$ or $5E12^+$ cells in the culture medium;

(c) culturing the progeny of the isolated $AC133^+$ or $5E12^+$ cell under conditions in which the isolated $AC133^+$ cell or $5E12^+$ cell differentiates to neurons, astrocytes, and oligodendrocytes; and

(d) detecting the presence of neurons, astrocytes, and oligodendrocytes, wherein the presence of neurons, astrocytes, and oligodendrocytes characterizes the isolated $AC133^+$ cell or $5E12^+$ cell as an NS-IC.

41. The method of claim 40, wherein the conditions in which the isolated $AC133^+$ or $5E12^+$ cell progeny differentiates to neurons, astrocytes, and oligodendrocytes comprises culturing the progeny of the isolated $AC133^+$ or $5E12^+$ cell on a laminin-coated surface in culture medium with fetal bovine serum (FBS) and without mitogenic growth factor.

42. A method for identifying the presence of a growth factor that affects the growth of a neurosphere initiating stem cell (NS-IC), the method comprising:

(a) combining a composition suspected of containing at least one growth factor that affects the growth of NS-IC with a composition comprising NS-IC, wherein the NS-IC are characterized as $AC133^+$ or $5E12^+$, $nestin^+$, and capable of differentiation to neurons, astrocytes, and oligodendrocytes lineages; and

(b) determining the growth of the NS-IC as a function of the presence of at least one of the growth factors in the composition, wherein an altered NS-IC growth, as compared with growth of NS-IC that have not been contacted with the composition suspected of containing at least one growth factor that affects the growth of NS-IC, indicates the presence in the composition of a growth factor that affects the growth of NS-IC.

43. The method of claim 42, wherein the growth factor is a neural survival factor.